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Context: Since the discovery of functional brown adipose tissue (BAT) in adult humans, there has been a renewed interest in the physiology of human BAT. Imaging studies from our laboratory and others have shown increased glucose uptake in adipose tissue regions assumed to be BAT in humans. We have also shown that human BAT from the supraclavicular (SCV) region is positive for uncoupling protein-1. To date, however, the oxidative capacity of this adipose tissue (AT) depot has not been characterized in humans.

Objective: We hypothesize that oxidative capacity is increased in the AT of the SCV region known to contain human BAT.

Design: This was an observational prospective cohort study.

Setting: The study was conducted at a referral center.

Patients: Participants were 13 patients for whom thyroid gland surgery was indicated.

Main Outcome Measure: Basal cellular oxygen consumption in human AT biopsy samples from the SCV region, known to be [^{18}F]fluorodeoxyglucose positron emission tomography-computed tomography–positive, was compared with the cellular oxygen consumption in subcutaneous white adipose tissue (WAT) from the same region of the same subject.

Results: We show for the first time that AT from the human BAT region displays increased oxygen consumption ($P < .05$), on average 300% higher, than subcutaneous WAT of the same individual. The contribution of the proton leak to maximal respiration increased with age in the WAT but not in the AT from the BAT region.

Conclusions: These results suggest that human adipose tissue from the BAT region can be distinguished from subcutaneous WAT by a higher basal oxidative capacity. Additional studies are warranted to further elucidate the metabolic and bioenergetic characteristics of this AT depot in humans. (***J Clin Endocrinol Metab*** **98: E1230–E1234, 2013)**

Since the discovery of functional brown adipose tissue (BAT) in adult humans (1, 2), scientific interest in the physiology of human BAT has dramatically increased. Positron emission tomography and computed tomography studies using the glucose tracer [^{18}F]fluorodeoxyglucose have shown that cold-induced BAT activity is inversely related to body fat percentage, and subjects with high glucose uptake in BAT also have increased thermogenesis (1, 3, 4). In addition, other studies have used various metabolic tracers to measure in vivo BAT activity (5,

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Abbreviations: AT, adipose tissue; BAT, brown adipose tissue; FCCP, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone; NE, norepinephrine; SCV, supraclavicular; UCP-1, uncoupling protein-1; WAT, white adipose tissue.

6). Taken together, these studies show increased metabolic activity in an anatomical region presumed to contain BAT in humans, located in the adipose tissue (AT) depot dorsal from the neck muscles and referred to as the supraclavicular (SCV) region. To date, AT in this region has not been characterized in great detail ex vivo, although some studies have shown the presence of uncoupling protein-1 (UCP-1) in preadipocytes from this AT (2, 5, 7). We recently showed that adipocytes from this region can in fact be classified as "beige" fat cells, adipocytes genetically distinct from white and brown adipocytes, but possess the capacity to induce high levels of UCP-1 expression as do brown adipocytes (8).

Here we had the unique opportunity to characterize perioperative human AT biopsy samples taken from the SCV region, which is now recognized to contain brown and beige adipocytes. It is important to mention that oxygen consumption measurements in AT are not trivial, and few reports describe respiration measurements in human white adipose tissue (WAT) (9, 10). In this study, we aimed to determine whether the AT from the BAT region could be distinguished from subcutaneous WAT of the same region based solely on oxidative capacity. To this end, we measured cellular oxygen consumption in these small AT samples of BAT and compared it with the subcutaneous WAT taken from the same region in the same subject. We hypothesized that oxygen consumption would be higher in the AT region known to contain human BAT than in the WAT of the same region.

Materials and Methods

Study protocol

Approval was obtained from the medical ethics committee of the Maastricht University Medical Center. Thirteen patients for whom thyroid gland surgery was indicated (indications are provided in Supplemental Table 1 published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org) gave written informed consent for perioperative AT biopsy samples. All subjects were euthyroid to rule out any influence of thyroid hormone (TSH mean, 2.1 ± 1.4 mU/L; range, 0.3–4.9 mU/L; normal TSH assay range, 0.4–4.3 mU/L) (Supplemental Table 2).

Biopsy procedure

AT biopsy samples for BAT and subcutaneous WAT from the SCV region were performed as described previously (1) (Supplemental Material).

Tissue processing

All tissue biopsy samples were immediately placed in a tube with warm (37°C) PBS. One portion each of the AT from the BAT and the WAT regions (±10% of total biopsy) was snap-frozen and stored at −80°C for future analyses. Details of the sample preparation for subsequent respiration measurements are provided in Supplemental Material.

Respiration measurements

To determine oxygen consumption of AT from the BAT region and WAT, high-resolution respirometry was performed at 37°C by polarographic oxygen sensors in a two-chamber Oxygraph (OROBOROS Instruments, Innsbruck, Austria). Baseline respiration, state 4, and state U were measured according to Hoeks et al (11). After completion of respiration measurements, chamber contents were stored at −80°C. Respiration results were normalized to the DNA content measured spectrofluorometrically in these chamber contents as described previously (12).

Statistical analyses

Statistics were analyzed using PASW Statistics (version 18.0, Chicago, Illinois). Respiration measurements within subjects were compared using a paired Student *t* test. A value of $P < .05$ was considered significant. The data are presented as means ± SEM.

Results

Presence of UCP-1 in AT biopsy samples

In AT samples from 10 of the 13 subjects, additional immunofluorescence staining for UCP-1 was performed. All AT biopsy samples from the BAT region were UCP-1–positive, confirming the presence of brown adipocytes in this AT depot. UCP-1 immunofluorescence for the WAT biopsy samples was negative, confirming the absence of UCP-1 in the subcutaneous WAT biopsies.

AT respiration in AT from the BAT region compared with that in WAT

Figure 1 shows the respiration levels of subcutaneous tissue (WAT) and AT from the BAT regions. Basal respiration (without addition of substrates) was more than 3 times higher in AT from the BAT region than in WAT (430.9 ± 113.8 vs 132.6 ± 90.8 pmol of O_2 per s per mg of DNA, $P = .017$) (Figure 1). After addition of oligomycin to induce state 4 ("leak") respiration, oxygen fluxes for both AT from the BAT region and WAT significantly decreased (basal vs state 4; AT from the BAT region: 430.9 ± 113.8 vs 364.1 ± 102.0 pmol of O_2 per s per mg of DNA, $P = .002$; for WAT: 132.6 ± 25.2 vs 104.6 ± 21.8 pmol of O_2 per s per mg of DNA, $P < .001$). Upon titration of carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) oxygen fluxes increased to a maximally uncoupled state of respiration (state 4 vs state U; for AT from the BAT region: 364.1 ± 102.0 to 491.1 ± 117.5 pmol of O_2 per s per mg of DNA, $P < .001$; for WAT: 104.6 ± 21.8 to 189.4 ± 34.2 pmol O_2 per s per mg of DNA, $P = .003$). Although both tissue depots exhibit the same significant patterns in their responses to the inhibitor and uncoupler, AT from the BAT region is significantly higher in all 3

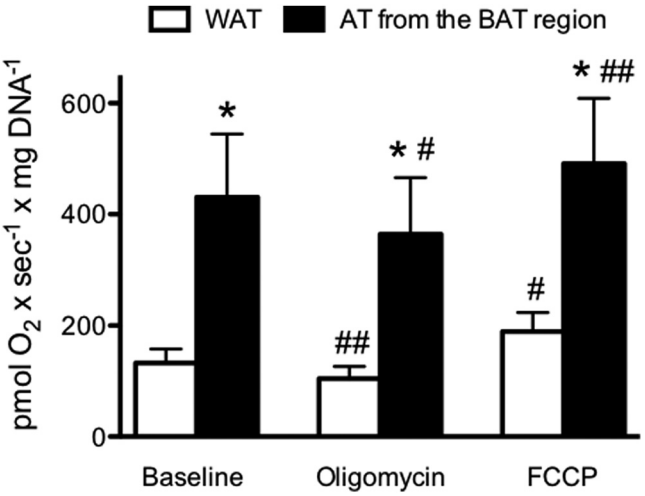

Figure 1. Cellular respiration levels of human WAT and AT taken from regions shown to contain BAT. Oxygen consumption in WAT and AT from the BAT region shown for basal respiration, oligomycin-insensitive leak state 4 respiration upon addition of oligomycin, and maximally uncoupled state U respiration after addition of FCCP. All uncorrected oxygen fluxes (expressed as picomoles of O_2 consumption $\times$ $second^{-1}$) for both AT from the BAT region and WAT were normalized for the DNA content in chamber solutions (milligrams per milliliter). Values shown are means $\pm$ SEM. *, $P < .05$ for comparison between AT from the BAT region vs WAT at basal, oligomycin-stimulated, and FCCP-stimulated oxygen fluxes, respectively. #, $P < .05$; ##, $P < .001$ for comparison between basal vs oligomycin and oligomycin vs FCCP-stimulated oxygen fluxes within tissues for WAT and AT from the BAT region.

states of respiration compared with the WAT from the same region (AT from the BAT region vs WAT, basal: $P = .017$; state 4: $P = .020$; state U: $P = .010$).

Age differences in respiration control ratios

The relative contribution of state 4 to state U respiration in WAT was significantly related to age ($r^2 = 0.466$, $P = .010$) (Figure 2). However, the ratio of state 4 to state U was not significantly related with age for the AT from the BAT region ($r^2 = 0.164$, $P = .170$) (Figure 2).

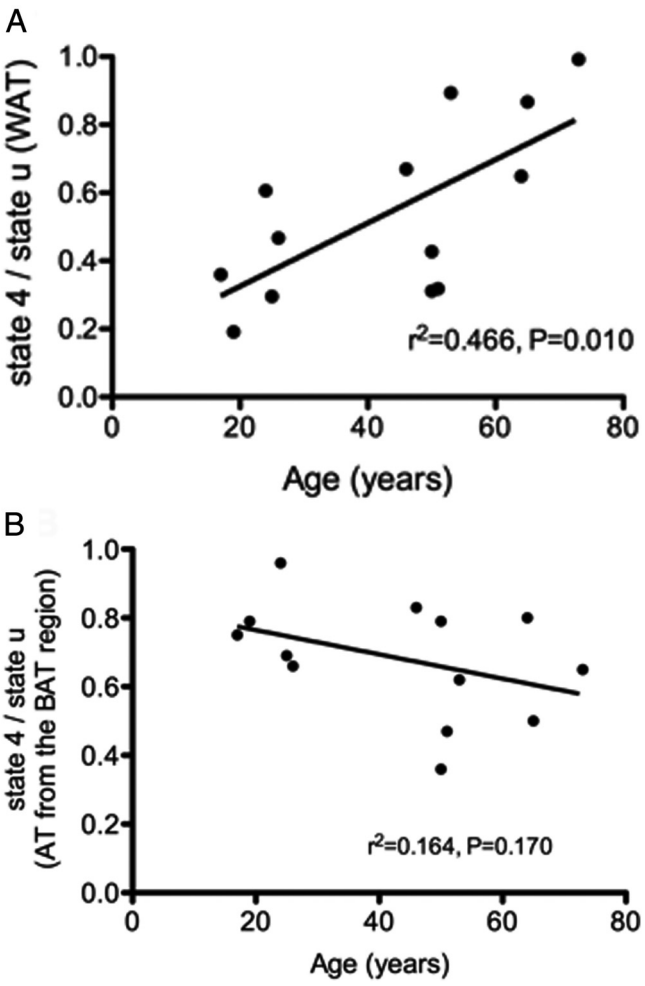

Figure 2. Relationships between subject age and mitochondrial respiration states of WAT and AT from the BAT region. A, Relationship between age and the relative contribution of state 4 leak respiration to maximally uncoupled state U respiration in WAT. B, Relationship between age and the relative contribution of state 4 leak respiration to maximally uncoupled state U respiration in AT from the BAT region.

Discussion

In the present study, oxygen consumption in human AT from the BAT region was compared with that of subcutaneous WAT taken from the same anatomical region of the same individual. We show here for the first time that respiration in AT from the BAT region is on average 3 times higher than in subcutaneous WAT. These findings are in line with previous animal studies demonstrating that oxidative capacity in BAT is relatively high (13). A recent study by Ouellet et al (6) showed that cold exposure increased [^{11}C]acetate uptake by ~150% in BAT, whereas another study showed that BAT perfusion (using [^{15}O]H_2O) increased ±200% and glucose uptake (using [^{18}F]fluorodeoxyglucose) increased ±600% in response to cold. These same parameters did not change significantly in WAT, and baseline BAT oxygen uptake and perfusion were already twice those of WAT (5, 6).

Here we show that AT taken from the region now identified to contain BAT consumes 300% more oxygen in the basal state and thus displays increased cellular respiration, compared with the subcutaneous WAT from that same region. This elevated basal respiration is markedly greater than the 10% to 20% elevation in basal respiration that has been reported in visceral WAT vs subcutaneous WAT (9). Our results indicate that AT from the BAT region is a highly metabolically active AT depot in humans.

It is important to note that biopsy samples from the SCV region in humans do not consist solely of brown or beige adipocytes, as may be the case in more homogeneous BAT depots in rodents. Rather, as observed in our Supplemental Figure 1 and other studies (14), the adipose

tissue of this region is a mixture of white and UCP-1–positive adipocytes, with a varying composition among subjects. Oxidative capacities of "pure" brown/beige adipocytes are probably much higher than what we find in heterogeneous depots in humans, thus highlighting the important need for development of strategies to induce and/or activate human brown/beige adipocytes such that the metabolic activity potential of this AT depot may be enhanced.

Because of the limited amount of human AT available and the amount required for respiration studies, we could not perform a further characterization of the AT from the SCV region, such as mitochondrial content, which most likely explains the difference in basal metabolic activity. It is generally thought that the main component of heat production (extra oxygen consumption) in BAT derives from UCP-1–mediated "leak" respiration (15). UCP-1–mediated thermogenesis needs to be activated by external stimuli, eg, a sympathetically induced norepinephrine (NE) release. In mice, maximal respiration in BAT upon addition of NE increases ~8-fold over basal respiration (13) and thus is much higher than the FCCP-induced respiration in our study. Unfortunately, we were unable to determine NE-stimulated mitochondrial respiration in the AT from the BAT region. Initial attempts were unsuccessful, and the limited amount of this valuable human tissue did not allow for further optimization of the procedure. In that respect, it is also known from rodent studies that BAT from several (eg, 6–8 mice) animals must be pooled to render enough isolated brown adipocytes that respond to NE ex vivo. Nevertheless, our basal respiration levels in AT from the BAT region, together with the fact that this tissue is heterogeneous with respect to white and brown/beige fat cell composition, suggest that true respiration in pure human brown/beige adipocytes may be high.

Interestingly, the contribution of state 4 to state U in WAT significantly increased with age, a finding that was not upheld in the AT from the BAT region. Mitochondrial dysfunction is known to be associated with aging and obesity (16). It is therefore possible that the maximally uncoupled state U (which is representative of the electron transport chain capacity) is impaired in WAT in older and obese subjects. However, we did not observe statistically significant relationships between age and BMI with state U; thus, further research is warranted to explore the possible effect of age and obesity on the mitochondrial capacity in WAT.

Recent reports show that within WAT there is a subpopulation of cells that have the capacity to become thermogenic (17–19). These "beige" or brite (brown-in-white) cells possess UCP-1 and are thus capable of thermogenesis upon sufficient stimulation (17–19). Intriguingly, we recently showed that AT taken from the same area as in this report in fact is composed of such beige cells, which are genetically distinct from white and brown fat (8). Respiration analyses of cloned beige cells showed that respiratory activity of these cells are comparable to classic brown adipocytes (8). These findings suggest that the amount of BAT in humans may be inducible when activated by the right stimulators, consistent with our recent finding of induction of human BAT after weight loss (20).

In summary, we here show that the basal oxidative capacity of human AT from the region characterized as BAT can be distinguished from subcutaneous AT by ~300% higher oxygen consumption. This illustrates that AT from the human BAT region is a distinct adipose tissue depot with high mitochondrial activity. Future studies should be directed to further elucidating the metabolic and bioenergetic aspects of this complex AT depot in humans. Cultured adipocytes from this depot may be of specific interest to identify molecular targets that are able to stimulate this tissue. Such studies could play an important role in obesity treatment by inducing BAT activity and increasing energy expenditure.

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